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Comparison of Effects of Famciclovir and Valaciclovir on Pathogenesis of Herpes Simplex Virus Type 2 in a Murine Infection Model

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The effects of famciclovir (FCV) and valaciclovir (VACV) were compared in a cutaneous infection model for herpes simplex virus type 2 (HSV-2). The compounds were administered orally from day 1 to day 5 postinfection. Both compounds reduced local inflammation and virus replication in the skin. FCV markedly reduced mortality and virus replication in the nervous system. On the cessation of therapy after 5 days, when the levels of infectious virus in the tissues were reduced to below the level of detection, there followed a rebound of virus replication in the ganglia and brain stems of mice that had been treated with VACV. The recurrence of infection in the brain stem occurred on three separate occasions. No such recurrences were observed following FCV treatment. When ganglia were explanted from survivors 6 weeks later, latent virus was shown to be reactivated in all 10 of 10 control, untreated mice. The number of mice whose ganglia yielded virus was reduced to 60% in mice that had been treated with VACV, whereas no mice that had been treated with FCV had evidence of latent infection by this test.

Famciclovir (FCV) and valaciclovir (VACV) are oral prodrugs of penciclovir (PCV) and aciclovir (ACV), respectively (2, 21, 23, 24). In a previous study we compared the effects of VACV and FCV in a murine cutaneous infection model for herpes simplex virus type 1 (HSV-1) in which mice were also immunosuppressed by means of cyclosporin A (8). The experiments described in that report showed that both compounds were effective in the model when therapy was commenced shortly after virus inoculation (8) or when therapy was delayed for 5 days before commencing treatment (9). However, a novel finding to emerge from the previous study was the reappearance of infectious virus in skin and neural tissues shortly after the cessation of VACV therapy. In marked contrast, no such recurrence of infection was seen in any FCV-treated animals. The rebound of infectious virus following VACV therapy occurred irrespective of the duration of treatment, which was for 5 or 10 days, and similar results were seen in immunocompetent animals (10, 19).

The present study concerns the use of the same two compounds, but the virus used to produce the murine infection was a strain of HSV-2, and in the present study, no immunosuppression was applied. Ideally, a genital infection would have been used. However, we chose to use a cutaneous infection, which allowed highly quantitative measurements of the acute infection and the assessment of latency to be made. The results obtained following oral dosing with FCV or VACV confirm and extend those reported previously for HSV-1. Furthermore, striking differences were also observed in the ability to reactivate HSV-2 from ganglion explants after several weeks had elapsed following therapy with the two different compounds. These results are discussed in relation to possible differences in their mechanisms of action.

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MATERIALS AND METHODS

Virus strain and tissue culture. The virus used in the study was HSV-2 Bry (11, 20). Virus working stocks were grown in BHK-21 cells as described previously (9), except that virus isolation from tissue samples was carried out with medium containing 5% rather than 2% newborn bovine serum (NCS).

Mice and virus inoculation. BALB/c female mice (Bantin & Kingman, Kingston, Hull, United Kingdom) were purchased at 3 to 4 weeks of age and were inoculated 1 week later. The virus suspension (2×10^4 PFU/10 μl) was inoculated into the skin of the left ear pinna.

Antiviral compounds and treatment regimen. FCV and VACV were synthesized at the laboratory of SmithKline Beecham by previously published methods (2, 12). The activities of ACV and PCV, which are the active metabolites of VACV and FCV, respectively, were measured by means of plaque reduction in BALB/c mouse 3T3 cells. The compounds were dissolved in double-distilled deionized water to give a dose of 50 mg/kg of body weight per mouse in 0.1 ml. Therapy was initiated at 22 h postinfection (p.i.) and was continued until day 5.5 p.i. The compounds were administered by oral gavage twice daily, at 8 a.m. and 6 p.m.

Measurement of clinical signs. All mice were assessed subjectively once per day. Groups of eight mice each were weighed individually, and deaths were recorded and skin thickness was measured for individual mice by means of an engineer's micrometer screw gauge. These methods have been described in detail previously (17). Ear lesions were defined as one or more vesicles (visible to the naked eye), blisters, crusts, or scabs. This did not include erythema or swelling, which were recorded as separate signs. Mice with flaccid ear pinnae (which failed to respond to a gentle stimulus) were recorded as showing "ear paralysis," and this was recorded separately. Other signs such as circling, monolateral and bilateral hind limb paralysis, and unsteady gait were recorded as "neurological signs."

Titration of virus in tissue samples. Tissue samples (ear pinna, brain stem, or the ipsilateral trigeminal ganglia) were obtained not less than 6 h after administration of the last dose of FCV or VACV. On each occasion tissue samples were obtained from three mice, and their tissues were tested independently. Tissues were minced with scissors and were ground in 1 ml of Eagle's minimum essential medium (EMEM) in glass homogenizers. The samples were subjected to a 1-min ultrasonic vibration in an ice bath. Debris was removed by slow-speed centrifugation, and the supernatant was diluted 10- and 100-fold in EMEM. Then, samples (0.2 ml) of the supernatant were inoculated onto BHK-21 cell monolayers, adsorbed for 1 h, and overlaid with EMEM containing 2% carboxymethyl cellulose to prevent the formation of secondary plaques, and virus plaques were checked daily and counted after 5 days of incubation. Coded numbers were assigned to samples for titration, and the results were read by a blinded investigator.

Reactivation of latent virus. The surviving mice in each treatment group were kept until 6 weeks p.i. Ten mice per treatment group were killed over a period of 4 days, and the contralateral and ipsilateral trigeminal ganglia and cervical dorsal root ganglia were explanted. Trigeminal ganglia were tested independently, but the dorsal root ganglia (CII, CIII, and CIV) were pooled for each side. The ganglia were incubated in a humidified atmosphere containing 5% CO_2

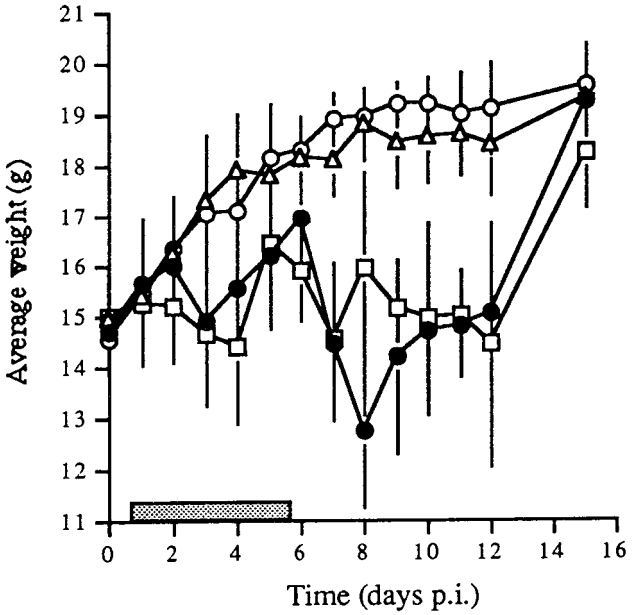

FIG. 1. Effect of antiviral therapy on weight gain during the acute phase of an HSV-2 infection. All mice were inoculated into the skin of the left ear pinna with 2×10^4 PFU of HSV-2. Antiviral therapy was administered twice daily by oral gavage at a dose of 50 mg/kg per dose from 22 h p.i. to day 5.5 p.i. inclusive. Eight mice were weighted at each time point. The horizontal bar represents the period of chemotherapy. Vertical bars are standard deviations. ●, infected controls; △, FCV-treated mice; □, VACV-treated mice; ○, uninoculated controls; ▨, time of therapy.

in 0.5 ml of EMEM containing 5% NCS as described previously (6, 10, 19), except that the ganglia were incubated for 10 days rather than the 5 days described previously. The samples were then ground in glass homogenizers, sonicated for 1 min in an ice bath, and spun at slow speed to remove debris. The supernatant was then diluted 10-fold, and 0.2 ml of the neat supernatant and 0.5 ml of the 1:10 dilution were inoculated onto confluent BHK-21 cell monolayers. Samples were adsorbed for 1 h and were then overlaid with EMEM containing carboxymethyl cellulose. Virus plaques were counted after 5 days. All samples were coded and read by a blinded investigator as described above.

Experimental design. A total of 280 mice were inoculated into the left ear pinna with 2×10^4 PFU of HSV-2 and were treated as follows: (i) no chemotherapy (n = 80), (ii) FCV at 50 mg/kg twice daily from 22 h p.i. to day 5.5 p.i. ($n = 50$), and (iii) VACV at 50 mg/kg twice daily from 22 h p.i. to day 5.5 p.i. ($n = 50$).

All mice were examined daily for clinical signs including weight gain and ear thickness increase. Three mice per group were killed on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 15, and the ear, brain stem, and left trigeminal ganglia were titrated for infectious virus. Separate groups of mice were kept for assessing mortality and latency ($n = 40$ mice for the untreated controls and $n = 30$ mice for each of the two treatment groups). Ten mice per group were killed during the 6th week p.i., and the contralateral and ipsilateral trigeminal and cervical dorsal root ganglia (CII, CIII, and CIV pooled) were explanted and tested for latency as described above.

RESULTS

Antiviral activity in cell culture. PCV and ACV had similar activities against HSV-2 Bry in BALB/c mouse 3T3 cells; the 50% effective doses (± standard deviation) were 0.06 ± 0.01 and 0.05 ± 0.02 μg/ml, respectively. In BHK-21 cells, the cell line used to quantify virus in tissue samples, PCV was less active than ACV (50% effective doses, 0.1 ± 0.02 and 0.05 ± 0.01 μg/ml, respectively).

Inoculation of mice. Untreated mice showed a significant loss of weight for a period of 12 days following virus inoculation (Fig. 1). The VACV-treated mice showed a weight loss pattern which was not significantly different from that for the infected controls. In contrast, the weight gain in FCV-treated mice was similar to that in the uninoculated controls. By day 15 p.i., the surviving mice regained weight and weighed the same as uninoculated controls.

TABLE 1. Effect of antiviral therapy on clinical signs during the acute phase of the infection

Antiviral therapy[a]	% of mice with the following clinical signs:				Mean time to death (days)
	Ear lesions	Ear paralysis[b]	Other neurological signs	Death	
None ($n = 40$)	50	85	58	60	10.8
FCV ($n = 30$)	17	53	3	3	21
VACV ($n = 30$)	33	53	23	23	16

[a] Treated mice received 50 mg of drug per kg twice daily from days 1 to 5.5 p.i. n is the number of mice in each treatment group.
[b] Ear paralysis was recorded as a separate neurological sign.

Clinical signs. In the absence of treatment, 85% of the mice developed ear paralysis, while 50% had ear lesions (Table 1). Both compounds reduced the incidence of ear paralysis; however, FCV also markedly reduced ear lesions and reduced the occurrence of other neurological signs. Furthermore, FCV was more effective than VACV in that the incidence of visible ear lesions, neurological signs, and mortality was reduced to a greater extent (Table 1). Neither compound had a marked effect on the incidence of ear paralysis.

Ear thickness increase. Untreated mice developed an inflammatory response in the inoculated ear which resulted in a peak of an approximately 100% increase in ear thickness by day 6 p.i. No such thickening response was observed in FCV-treated mice (Fig. 2). The response among the VACV-treated mice was more variable, and some mice developed ear swelling, although the differences between the two drug-treated groups were not significant. Both compounds, however, reduced ear swelling significantly in comparison with that in the untreated controls.

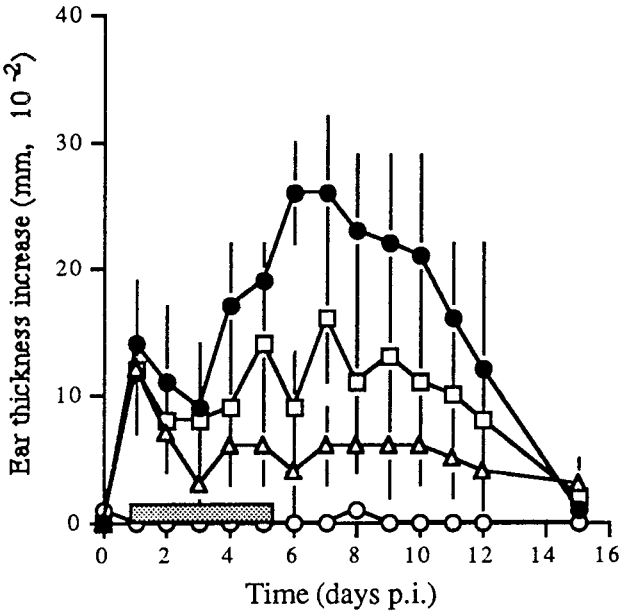

FIG. 2. Effect of antiviral therapy on virus-induced inflammation in the ear pinnae measured by means of ear thickness. Antiviral therapy was as described in the legend to Fig. 1. Eight mice were measured at each time point. The horizontal bar represents the period of chemotherapy. Vertical bars are standard deviations. Symbols are as defined in the legend to Fig. 1.

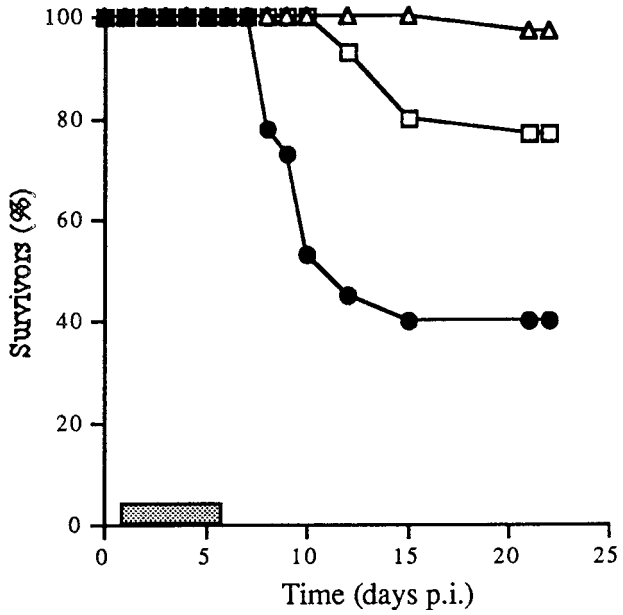

FIG. 3. Effect of antiviral therapy on the survival of mice inoculated with HSV-2 in the ear pinna and treated with FCV or VACV. Antiviral therapy was as described in the legend to Fig. 1. The horizontal bar represents the period of chemotherapy. Symbols are as defined in the legend to Fig. 1.

Mortality. In a group of 40 untreated mice, 24 mice (60%) died between days 8 and 15 p.i. Seven of 30 mice in the VACV-treated group died (23%), and death occurred between days 12 and 20 p.i. (Fig. 3). No mortality was observed in the FCV-treated group up to day 21 p.i., when one mouse died. This animal had shown neurological signs between days 10 and 12 p.i. The mean times to death were 10.8 days for untreated controls and 16 days for VACV-treated mice.

Virus growth in the ear. The titers of infectious virus in the control (untreated) mice peaked at day 2 p.i., and in the absence of chemotherapy, virus was cleared to undetectable levels by day 5 p.i. (Fig. 4). FCV accelerated the clearance of infectious virus to undetectable levels by day 3 p.i., while VACV-treated mice showed less of a reduction in the level of infectious virus, and although the titer of virus was significantly lower than that in the controls at day 2 p.i., the clearance of infection was not accelerated compared with that in the untreated controls.

Virus growth in the brain stem. In untreated mice, virus was first detected in the central nervous system (CNS) at day 4 p.i., and virus replication was sustained until day 9 p.i., whereafter it was cleared from survivors (Fig. 5). Neither compound prevented virus replication in the CNS, although both FCV and VACV reduced virus titers on days 4 and 5 p.i., and in FCV-treated mice, the level of virus in CNS tissue remained below the level of detection for the rest of the experiment. Initially, VACV reduced the titers of infectious virus (no virus was detected in the CNS on day 5 p.i. in VACV-treated mice), but on the cessation of therapy, there was a rebound of infectious virus on each of days 6, 8, and 12 p.i. On each occasion the titers of virus were closely similar among the three mice tested. Tissue samples obtained from all surviving mice tested after day 12 p.i. were uniformly negative. There was no recurrence of infectious virus in the brain stem of any mouse on the cessation of FCV therapy.

Virus growth in the trigeminal ganglia. In untreated mice, virus was first detected in the left trigeminal ganglia on day 4 p.i., where it peaked at approximately 10^3 PFU per ganglion. Infectious virus was detected in ganglia at all subsequent time points up to day 9 p.i. The virus titers in the trigeminal ganglia were reduced in mice treated with FCV or VACV compared with the titers in the controls, and virus was detected only at day 5 p.i. in mice treated with FCV (Fig. 6). In mice treated with VACV, however, there was a rebound of infectious virus following the cessation of VACV (but not FCV) therapy on day 6 p.i., when all three mice tested yielded closely similar virus titers.

The reactivation of virus from ganglia explanted from survivors. Ten mice per group were killed during the 6th week p.i.,

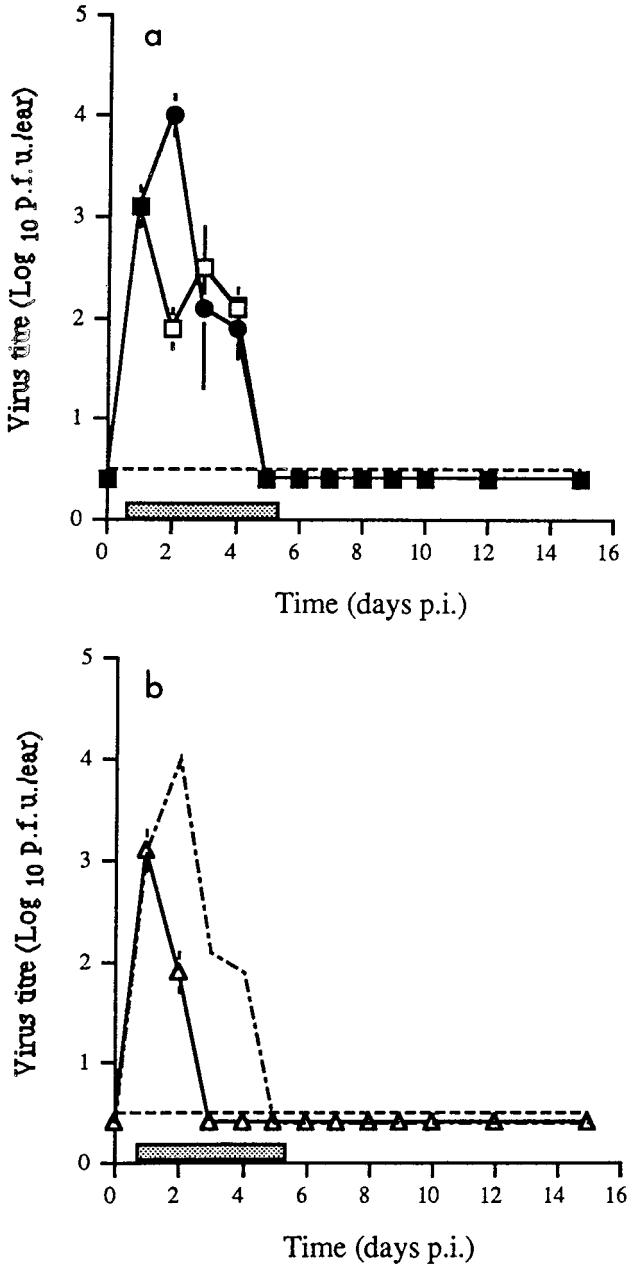

FIG. 4. Effect of antiviral therapy on the titers of infectious virus in the ear. Antiviral therapy was as described in the legend to Fig. 1. Three mice were tested at each time point. The horizontal bar represents the period of chemotherapy. Vertical bars are standard deviations. – – –, limit of sensitivity; — – —, infected control. The other symbols are defined in the legend to Fig. 1. (a) VACV-treated mice. (b) FCV-treated mice.

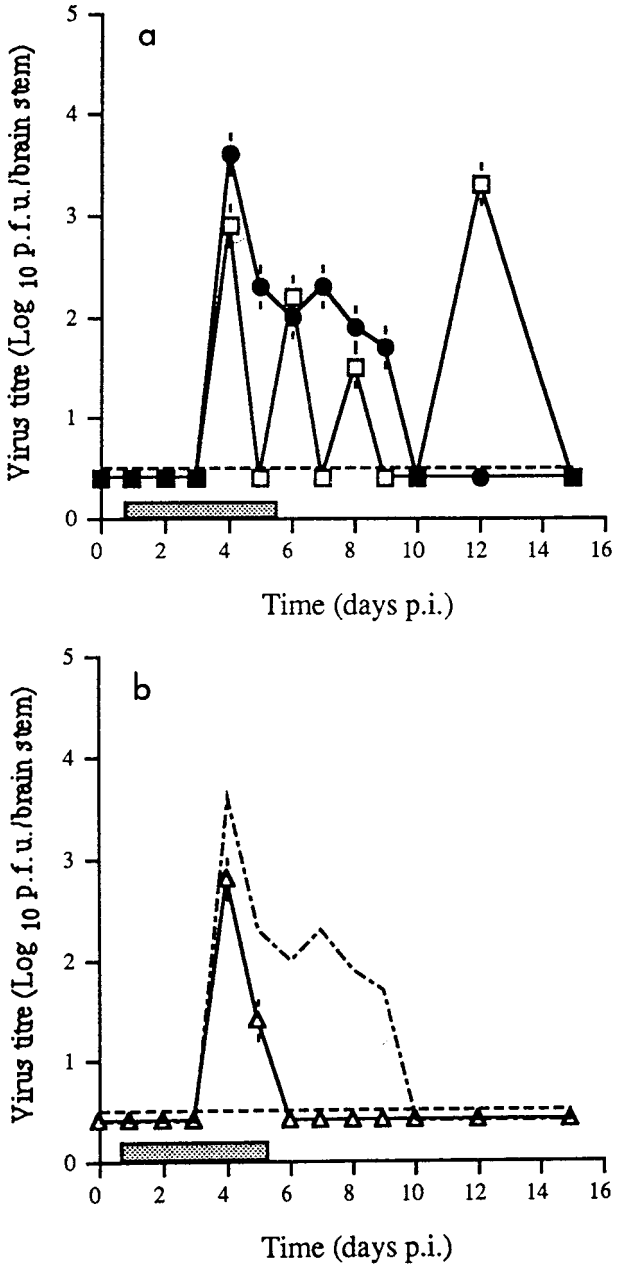

FIG. 5. Effect of antiviral therapy on the titers of infectious virus in the brain stem. Antiviral therapy was as described in the legend to Fig. 1. Three mice were tested at each time point. The horizontal bar represents the period of chemotherapy. Vertical bars are standard deviations. — — —, infected control; - - -, limit of sensitivity; the other symbols are defined in the legend to Fig. 1. (a) VACV-treated mice. (b) FCV-treated mice.

and the contralateral and ipsilateral trigeminal and cervical dorsal root ganglia were explanted. The ganglia were incubated for 10 days to allow reactivation to occur. The trigeminal and pooled dorsal root ganglia were tested independently for the presence of infectious virus. Among the survivors from the group that had received no chemotherapy, reactivated virus was recovered from all 10 of 10 mice tested. All left trigeminal, dorsal root, and right trigeminal ganglia were positive for virus, and 6 of 10 of the right dorsal root ganglia were also positive. The results (Table 2) indicate a marked difference between the control mice and mice that had been treated with the antiviral compounds. For mice that had been treated with VACV, virus from 5 of 10 of the left and right trigeminal ganglia and 6 of 10 and 3 of 10 of the left and right dorsal root ganglia, respectively, was reactivated. There were, however, no positive samples from any of the 10 FCV-treated animals tested. There was a close correlation between the results for trigeminal and dorsal root ganglia from individual mice for both treatment regimens.

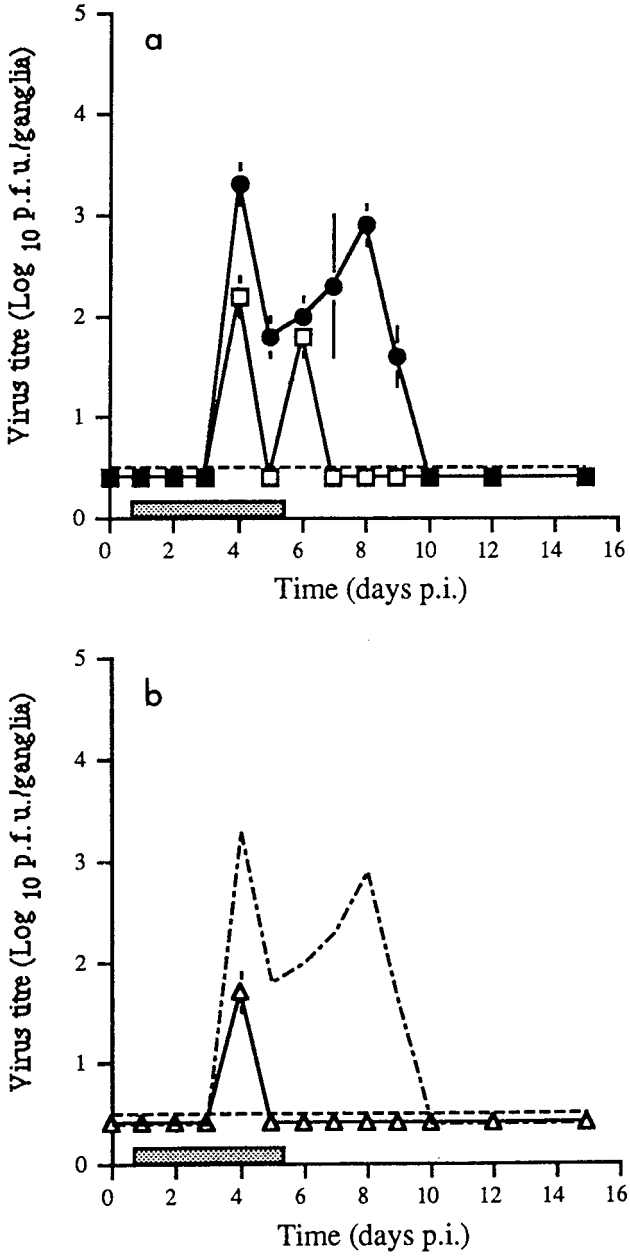

FIG. 6. Effect of antiviral therapy on the titers of infectious virus in the ipsilateral trigeminal ganglia. Antiviral therapy was as described in the legend to Fig. 1. Three mice were tested at each time point. The horizontal bar represents the period of chemotherapy. Vertical bars are standard deviations. — — — — —, infected control; - - -, limit of sensitivity; the other symbols are defined in the legend to Fig. 1. (a) VACV-treated mice. (b) FCV-treated mice.

TABLE 2. Proportion of mice from which virus was reactivated from explanted ganglia at 6 weeks p.i.[a]

Group	No. of mice with positive ganglia/no. of mice tested[b]				% Mice yielding at least one positive ganglion
	Left T/G	Right T/G	Left CDR	Right CDR	
Untreated control	10/10	10/10	10/10	6/10	100
FCV	0/10	0/10	0/10	0/10	0
VACV	5/10	5/10	6/10	3/10	60

[a] Antiviral therapy was as described in the legend to Fig. 1.
[b] T/G, trigeminal ganglia; CDR, cervical dorsal root ganglia.

DISCUSSION

Pharmacokinetic data reported previously (8) suggest that the levels of ACV and PCV in blood (which are products of VACV and FCV, respectively) are likely to be similar. In summary, following oral administration of a single 50-mg/kg dose of FCV or VACV to BALB/c mice, the blood concentration-time curves for PCV and ACV were almost identical, leading to very similar areas under the curve. Comparison of these data with those obtained following 5 days of dosing showed that there was no significant alteration in blood PCV or ACV levels. No residual PCV or ACV (<0.2 μg/ml) was detected in blood from 8 h onward after the administration of the last dose (8).

When the compounds were tested against HSV-2 in vitro, there was little difference in the antiviral activity measured in murine cells to account for any differences in their effects on virus pathogenesis in vivo; however, it is well established that the intracellular half-life of PCV triphosphate is much greater than that of ACV triphosphate (21), and this could help to explain the differences observed in the present study. Moreover the distribution of ACV or PCV to the CNS compartment has not been investigated. If there are differences this is also likely to be an important factor in controlling virus infection within the murine nervous system.

In general terms, FCV was much more effective in reducing virus replication and modulating clinical signs than VACV in this particular model. Specifically, the three most striking findings to emerge from this study were (i) the effects of FCV on weight gain and mortality, (ii) the recurrence of infectious virus in neural tissues after the cessation of VACV therapy, and (iii) the failure to reactivate virus from ganglion explants following FCV therapy.

The reduced weight gain observed in infected mice has not been fully elucidated. It may result from dehydration, and this probably reflects the loss of water since the daily intake measured by the water consumed showed very little variation except on day 8 p.i., when mice drank significantly less. There was no crystalluria, and uninfected mice treated with either compound showed no weight loss (or any other signs of toxicity). The rapid return to normal weight in all infected mice on day 12 and in the FCV-treated mice from day 2 appears to be an accurate measure of the general well-being of the animals, which concurrently appeared brighter and less ruffled.

Neither compound was particularly effective at reducing virus replication in the ear pinna. The levels of infectious virus were generally less than those observed following HSV-1 inoculation (11), and it was of interest that virus was cleared from all mice including untreated controls before neurological signs and deaths occurred. The clinical signs observed in the infected mice are thought to relate more closely to the spread of virus into neurological tissue, and it is presumed that the reduction of virus replication in central neural tissue was directly related to the improved survival of FCV-treated mice. VACV therapy produced a reduction in virus titers in the nervous system during chemotherapy, and these reductions were highly significant on days 4 and 5 p.i. The mean time to death was increased compared with that for controls, and there was a reduction in overall mortality, although this was less than that observed in FCV-treated mice.

The recurrence of infectious virus in neurological sites following the cessation of therapy confirms similar observations previously reported for HSV-1 (8–10, 19). Moreover, it has been noted that in tissue cultures infected with HSV-1, infectious virus replication remains suppressed following treatment with PCV for 18 h. Conversely, viral replication rapidly resumed after removal of extracellular ACV (1, 4). In addition, Sutton and Boyd (18) have reported that PCV treatment, but not ACV therapy, led to prolonged suppression of virus replication on the cessation of therapy of an intraperitoneal HSV-1 infection in mice. The absence of such a recurrence of infectious virus in FCV-treated mice may be a reflection of the more sustained levels of PCV triphosphate that are believed to exist in HSV-1 or HSV-2-infected cells (5, 22).

The failure to reactivate virus from explanted dorsal root and trigeminal ganglia is striking. The most likely explanation for this is that the compounds reduced virus replication during the acute phase of the infection, leading to less exposure of neural tissue to infectious virus and resulting in the establishment of fewer foci of latent infection. The reduced level of reactivation may therefore simply reflect a reduction in the number of latently infected neurons to below the sensitivity of the assay. This explanation has been invoked in previous studies on the effects of nucleoside analogs to explain the reduced level of establishment of latency (3, 6, 16). It is generally held that ACV therapy can only protect mice from the establishment of latency for a short period after infection, possibly as little as 6 h (7, 13–16). However, in a previous experiment it was shown that FCV reduced the incidence of latency significantly for HSV-1 in this model, although the start of treatment was delayed for 5 days (10, 19). It remains to be determined whether this reflects a quantitative or a qualitative change in the distribution of HSV-1 or HSV-2 genomes in the latently infected tissue.

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